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ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

The Effects of Hypoxia on Oxidative Stress, Inflammation, and DNA in Patients With Severe Sleep Apnea Syndrome

Ağır Uyku Apne Sendromlu Hastalarda Hipoksinin Oksidatif Stres, İnflamasyon ve DNA Üzerine Etkisi

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Öz

Amaç: Obstrüktif uyku apne sendromu, uyku sırasında tekrarlayan apne-hipopne ile ilişkili bir hastalıktır. Apne-hipopne atakları oksijen satürasyon seviyelerini düşürerek hipoksiye neden olur. Hipoksinin neden olduğu oksidatif stres, inflamasyona ve DNA hasarına yol açabilir. Bu çalışmanın amacı şiddetli OSAS hastalarında aralıklı hipoksinin oksidatif stres, inflamasyon ve DNA'ya olan etkilerini araştırmaktır. **Gereç ve Yöntem:** Gece boyunca toplam 67 birey polisomnografi ile ölçüldü. Periferik ven kanı toplandı. Kan serumunda kolesterol, trigliserit, HDL, hs-CRP, TAS ve TOS değerleri kalorimetrik yöntemle ölçüldü. DNA hasarını araştırmak için Comet assay analizi yöntemi kullanıldı. **Bulgular:** Ağır OSAS hastalarında oksidatif stres ve inflamasyon parametrelerinde istatistiksel olarak anlamlı bir artış saptadık ($p < 0.05$). Bunun yanında, şiddetli OSAS hastalığının DNA hasarı üzerindeki etkisini tespit edemedik. ($p > 0.05$). **Sonuç:** Ağır OSAS hastalarında oksidatif stres ve inflamatuar belirteçlerin belirgin olarak arttığını gösterdi. Bununla birlikte, artan oksidatif strese rağmen DNA hasarının olmayışı oluşan hipoksinin DNA'ya hasar verecek kadar uzun süreli olmadığı ve tek başına DNA hasarı oluşturmadığı şeklinde yorumlanmıştır.

Anahtar Kelimeler: Obstrüktif uyku apne sendromu, oksidatif stres, DNA hasarı

Abstract

Object: Obstructive sleep apnea syndrome is a disease associated with recurrent apnea-hypopneas during sleep. Apnea-hypopnea attacks cause hypoxia by lowering oxygen saturation levels. Oxidative stress induced by hypoxia can lead to inflammation and DNA damage. The aim of this study was to investigate the effects of intermittent hypoxia on oxidative stress, inflammation, and DNA in severe OSAS patients. **Methods:** A total of 67 individuals were measured by polysomnography during the night. Peripheral ven blood was collected. Cholesterol, triglyceride, HDL, hs-CRP, TAS and TOS values of all subjects were measured in blood serum by calorimetric method. The comet assay method was used to investigate DNA damage. **Results:** We found a statistically significant increase in oxidative stress and inflammation parameters in severe OSAS patients ($p < 0.05$). Furthermore, we could not detect the effect of severe OSAS disease on DNA damage. ($p > 0,05$). **Conclusions:** Our results showed that oxidative stress and inflammatory markers changed markedly in severe OSAS patients. However, the absence of DNA damage

despite increased oxidative stress has been interpreted as hypoxia, which is not long enough to damage DNA and does not cause DNA damage alone.

Keywords: Obstructive Sleep Apnea Syndrome, Oxidative stress, DNA damage

Introduction

Obstructive sleep apnea syndrome (OSAS) is a disease characterized by upper respiratory tract obstruction resulting in recurrent hypoxia and reoxygenation during sleep. (1). The diagnosis and severity of OSAS is made by evaluating apnea-hypopnea index (AHI) obtained after polysomnography (PSG). As a result of polysomnography, AHI <5 simple snoring AHI> 30 is considered as severe OSAS (2). AHI measures the frequency of airflow reduction associated with airway constriction. The patient cannot breathe properly or exhale from the nose or the mouth. This interrupts normal sleep and affects the saturation of blood oxyhemoglobin (3).

The hypoxia that occurs in OSAS patients stimulates the sympathetic system leading to the formation of oxidative stress. Damage occurs primarily in endothelial function with the activation of cellular mediators (such as NF-κB, vascular endothelial growth factor, cytokines, C-reactive protein, and monocyte) (4-7). Oxidative stress is characterized by the imbalance between the production and destruction of reactive oxygen species. Reactive oxygen metabolites increase in the blood of OSAS patients and may cause damage at the cellular level (8). In addition, reactive oxygen metabolites may cause DNA damage related to chromosome aberrations (9).

C reactive protein (CRP) is encoded by the gene on the first chromosome and is synthesized from liver cells in response to tissue damage, infection, and inflammation. CRP mediates the uptake of LDL by macrophages, leading to the expression of intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) from endothelial cells. As a result, it causes endothelial dysfunction and atherosclerosis prepare the ground (10, 11).

In our study, we aimed to investigate the effect of oxidative stress level and inflammation on DNA damage in patients with severe OSAS in the light of this information.

Materials and Methods

Study Design

This study was a hospital-based patient-control study. All patients and controls were collected from Süleyman Demirel University Medical Faculty Hospital and Isparta City Hospital, Isparta, Turkey. Obstructive sleep apnea syndrome was suspected and individuals who applied to the chest diseases clinic were polysomnography during the night in the sleep laboratory.

Definitions

According to obtained apnea-hypopnea index (AHI); AHI <5 controls, AHI> 30 severe OSAS were accepted (2). Obesity and overweight are evaluated in body mass index (BMI). BMI is calculated as a function = Weight (kg) / [Height (m)]2. According to World Health Organization (WHO), BMI <18.5 is normally mild, BMI 18.5-24.9 normal, BMI 25-29.9 overweight and BMI> 30 obese (12). Oxidative stress index was calculated (OSI)= TOS, µmol H2O2 equivalent/L/ TAS, µmol Trolox equivalent/Lx100 (13).

Ethical considerations

This study was approved by the Ethical Committee of the Süleyman Demirel University, Medical Faculty (Resolution no. 190; dated October 26, 2017).

Sample Collection

After polysomnography, 10 ml of venous blood was taken with ethylene-diamine-tetraacetic-acid (EDTA) for use in comet assay method. For biochemical analysis, 4 ml of venous blood was taken with vacuum gelled tubes. The blood taken in the gelled biochemistry tube was centrifuged and serum was obtained.

Biochemical Analysis

Total antioxidant capacity (TAS), Total oxidant capacity (TOS), highly sensitive C-reactive protein (hs-CRP), cholesterol, triglycerides, high-density lipoprotein (HDL) were studied in serum with the Beckman Coulter AU5800 autoanalyzer on the same day.

TAS and TOS spectrophotometric methods were studied using Reel Assay Diagnostic Kits.

TAS principle: Antioxidant substances present in the samples were measured for colorless form reduction of the dark blue-green ABST radical solution, one of the reagents in the kit, and the absorbance change of the reaction mixture at 660 nm. Absorbance change is

directly proportional to the TAS level in the samples. The method was calibrated with a stable antioxidant standard solution (E vitamin analogue) Trolox Equivalent.

TOS principle: The oxidizing substances present in the samples oxidize the ferrous ion chelator complex to the ferric ion. The ferric ion form forms a colored complex with the chromium in the acidic medium. The color formation (as measured by the number of oxidant molecules present in the samples) was measured spectrophotometrically and the TOS value was obtained. The method was calibrated with hydrogen peroxide.

hs-CRP principle: Immune complexes formed in solution scatter light in proportion to their size, shape, and concentration. Turbidimeters measure the reduction of incidence light due to reflection, absorption, or scatter. In this procedure, the measurement of the rate of decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution is the result of complexes formed during the immunological reaction between the CRP of the patient serum and rabbit anti-CRP-antibodies coated on latex particles.

Cholesterol, triglycerides (TG), high-density lipoprotein (HDL) were studied spectrophotometrically. Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated according to the Friedwald formula (14). LDL = Total cholesterol - (TG / 5 + HDL), VLDL= TG/5

Detection of DNA damage by Comet assay method
The comet assay is based on the migration of DNA molecules/particles that have different electrical charges in alkaline pH. The level of migration indicates the level of damage to the DNA (15, 16).

Comet assay principle: In this method, blood samples were first slowly added to the histopaque in a ratio of 1: 1. Centrifugation was carried out at 2000 rpm for 20 minutes. After centrifugation, the turbid fraction formed in the supernatant of the leukocytes was removed, mixed with PBS at a ratio of 1: 1, and centrifuged at 2500 rpm for 10 minutes. The supernatant was then removed from the medium and the remaining cells were diluted with 25-30 µl PBS. Prepared at a temperature close to body temperatureCells mixed with LMA were spread over NMA jelly lamella, which had been precoated previously. After the cells were immersed in agarose gel on the slide, they were then allowed to stand for one hour in a lysis solution containing high concentrations of salt and detergent. During lysis, 10% dimethyl sulfoxide was added to the lysis solution to prevent free radical-mediated DNA damage due to iron, which was cleaved by the breakdown of erythrocytes present in blood and tissue specimens. The slides were washed several times with a suitable buffer to remove cellular debris, residual detergent, and salts. The preparations were incubated in dark and cold medium for 15-20 minutes in the electrophoresis buffer of high alkaline pH (pH> 13) to elucidate double-stranded DNA structure prior to electrophoresis. Electrophoresis was performed at 25 V and 300 mA for 25 minutes. The slides were stained using ethidium bromide. It was examined under a fluorescent microscope (Zeiss Imager A1). With the Comet method; tail length, tail moment and percentage of tail DNA were measured 400X magnification (Figure 1 A, B).

Statistical analysis
Normal distribution of all parameters was evaluated with One-Sample Kolmogorov-Smirnov Test. Statistical analysis was performed with independent sample t-test and Mann-Whitney U according to normality distribution. Pearson and Spearman's analyzes were used for correlation analysis. Statistical analysis was carried out with a commercial statistical package (IBM SPSS Statistics 24 software). P-values <0.05 were considered statistically significant.

Results

According to polysomnography results, 34 controls (AHI <5) and 33 severe OSAS patients (AHI≥30) were obtained. All subjects belonged to the Turkish population from Isparta. The characteristics of all the groups are summarized in Table 1.

Parameters of AHI, BMI, age, and oxygen-desaturation-index (ODI) were increased in the patient group and oxygen saturation (SaO2) was decreased. When evaluated in terms of lipid profile, there was an increase in TG and VLDL patient group, no difference in cholesterol, HDL and LDL values (Table 1).

The oxidative stress (TOS and OSI) and inflammation (hs-CRP) parameters were increased in the patient group and TAS was increased in the control group.

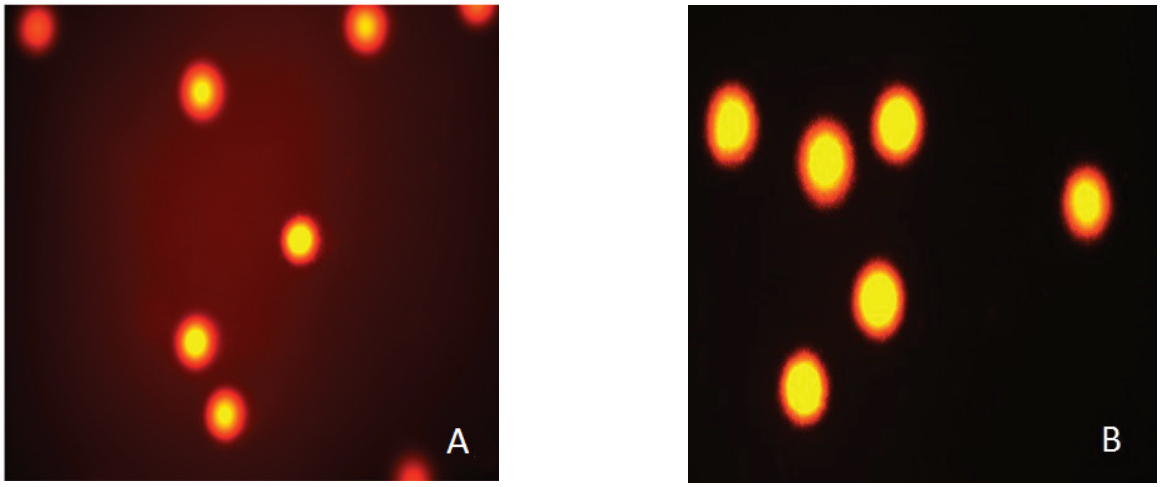

Figure 1 A) Control, **B)** Severe OSAS. Magnification: 400X.

Table 1 Demographic characteristics and of severe OSAS patients and control group (Mean ± SD)

Parameters	Control (n=34)	Patient (n=33)	P-value
Age	27,3±9,3	54,2±11,3	p<0,001
BMI	22,2±3,0	33,48±5,2	p<0,001
AHI	2,55±1,2	53,5±20,4	p<0,001
ODİ	2,62±1,2	51,5±3,5	p<0,001
SaO2	89,2±2,4	87,0±0,9	p=0,042
Cholesterol	185,8±6,4	202,8±9,8	0,154
TG	142,9±10,8	184,9±14,2	0,021
HDL	45,6±11,2	41,5±9,5	0,117
LDL	111,6±32,2	122,7±46,8	0,401
VLDL	28,5±12,7	36,9±16,3	0,022

Table 2 Statistical analysis of oxidative stress and DNA damage parameters (Mean ± SD)

Parameters	Control (n=34)	Patient (n=33)	P-value
TAS	2,06±0,9	1,17±0,2	0,02
TOS	15,1±5,1	46,8±24,7	p<0,001
OSİ	0,84±0,3	4,34±3,01	p<0,001
hs-CRP	1,8±1,4	7,1±1,7	p<0,001
Tail DNA	11,3±7,6	14,6±8,2	0,093
Tail DNA percent	4,4±4,0	4,5±2,3	0,070
Tail moment	1,6±0,2	2,82±2,80	0,144

Furthermore, when DNA damage was assessed, no differences were found in tail DNA, tail DNA percentage, and tail moment parameters (Table 2).

Discussion

In obstructive sleep apnea syndrome, apnea and hypopneas that develop during sleep are caused by hypoxia, which results in a decrease in oxygen saturation. The reduction in oxygen saturation results in the formation of reactive oxygen species (17). The resulting reactive oxygen species leads to cell damage with a damage similar to ischemia/reperfusion injury (18). The resulting intermittent hypoxia and oxidative stress cause damage to the DNA strand by breaking it (9). Several studies have been published on the effects of OSAS on oxidative stress, inflammation, and DNA damage. In 2003, Christou et al. have been found that antioxidant capacity decreased in patients with severe OSAS. Jordan et al. in their study in 2005, it was reported that oxidative stress in OSAS patients caused endothelial dysfunction leading to cardiovascular and cerebrovascular diseases (19). In 2007, Kontogianni et al., they found that basal DNA damage was increased and DNA repair capacity was reduced in OSAS patients (20). In 2014, Xie et al. DNA damage were investigated by micronucleus method in OSAS patients and it was found that DNA damage was significant (21). Unlike this study, Kang et al. In their study, they investigated oxidative stress with serum MDA levels in OSAS patients, DNA damage with the comet assay method, but could not achieve a meaningful result (22). The study on hs-CRP in OSAS patients in 2012 was not statistically significant (23). In 2014, Kim et al. showed a significant increase in hs-CRP levels in OSAS patients (24). In this literature, oxidative stress, inflammation and DNA damage in OSAS disease are not clear.

In our study, we found that hs-CRP, TAS, TOS and OSI values were statistically significantly higher when compared with severe OSAS patients and control group. We also found low TAS levels in patients with severe OSAS. We could not detect DNA damage in severe OSAS patients.

In conclusion, our study supports that OSAS disease contributes to the formation of oxidative stress and inflammation, but it does not support its effect on DNA damage. The absence of DNA damage despite the increased oxidative stress in severe OSAS patients suggests that oxidative stress resulting from internaiton hypoxia in OSAS does not start to cause DNA damage alone, but may lead to DNA damage due to other factors (smoking, drug use, etc.).

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